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#11
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Serial No: 09/593,914
Date Filed: June 14, 2000
Application Title: Probes, Probe Sets, Methods And Kits Pertaining To The
Detection, Identification And/Or Enumeration Of Yeast;
Particularly In Wine
Applicants: Hyldig-Nielsen et al.
Group Art Unit: 1655
Examiner: C. Myers
Action Date: July 18, 2001
Action Type: First Office Action On Merits - NON-FINAL
Certified Mail No.: 7099 3400 0007 5728 5682

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37 C.F.R. § 1.8

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Commissioner for Patents, Washington, DC 20231 on this 17th day of January, 2002.

Brian D. Gildea
Reg. No. 39,995

DECLARATION UNDER 37 C.F.R. § 1.131
TO OVERCOME A 35 U.S.C. §102(a) REJECTION

Commissioner for Patents
Washington, DC 20231

Dear Sir or Madam:

We, Jens J. Hyldig-Nielsen, of 1460 Highland Street, Holliston, MA, Heather P. O'Keefe of 248 Marrett Road, Lexington, MA and Henrik Stender of 137 Fox Road, #402, Waltham, MA do hereby declare and state that:

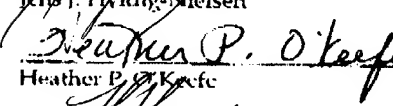
819901-US

1. We are the inventors of the above captioned patent application;
2. We are also identified as co-authors of the publication: Stender, H. et al., A new molecular method for simultaneous identification and enumeration of *Brettanomyces* in wine. Abstracts of the General Meeting Of The American Society For Microbiology, 99: 516 (1999) (copy attached as Exhibit A).
3. The invention as described and claimed in the present application was completed, based upon our work performed in the United States of America, prior to the publication date of said abstract;
4. The work described in said published abstract was derived from knowledge of our work based upon the participation of all of the identified authors in a program designed to produce a commercial product for the determination of *Brettanomyces* in wine;
5. The identified authors, other than ourselves, are not inventors of the subject matter claimed in the present application, but were identified as authors of the published abstract in conformance with professional standards used in determining authorship for publications.

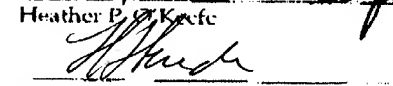
We further declare that all statements made in this Declaration are true and that all statements made on information and belief are believed to be true. Moreover, these statements were made with the knowledge that willful false statements and the like made by me are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.


Jens J. Haldig-Nielsen

01/17/02
Date


Heather P. O'Keefe

1/17/02
Date


Henrik Stender

1/17/02
Date

EXHIBIT A

P.D. ~~200~~ 31-05-1979
P. S16 ①
XP-000952544

P-26. A New Molecular Method for Simultaneous Identification and Enumeration of *Brettanomyces* in Wine

H. STENDER¹, H. PERRY-O'KEEFE¹, J.J. HYLDIG-NIELSEN¹, A. BROOMER¹, M. SARACINO¹, C. KURTZMAN², B. YOUNG³, J.M. COULL¹, ¹Boston Probes, Inc., Bedford, MA; ²Natl. Ctr. for Agric. Utilization Res., Peoria, IL; ³Millipore, Bedford, MA

Brettanomyces (ascosporic state of *Dekkera*) are well recognized as spoilage yeasts in wine causing 'mousiness', an undesirable odor and taste. Current methods for identification and enumeration take 1-2 weeks and rely on semi-selective culture medium followed by final identification from morphology and biochemical testing. A new *in situ* hybridization method using peptide nucleic acid (PNA) probes for simultaneous identification and enumeration of *Brettanomyces* within 2 days has been developed. The wine sample is filtered to isolate and separate individual microorganisms onto a membrane which is placed on a culture medium for up to 44 hours prior to testing. Microscopic colonies of *Brettanomyces* are detected on the membrane by hybridization with peroxidase-labeled PNA probes targeting *Brettanomyces* 26S rRNA. Excess probe is removed by washing and hybridized probe is visualized by a chemiluminescent reaction. Each *Brettanomyces* micro-colony is observed as a small dot providing simultaneous identification and enumeration. 100% sensitivity and 100% specificity of the probes have been determined using reference strains and wine isolates of *Brettanomyces* as well as other yeast species potentially found in wine. Results obtained using the method to detect *Brettanomyces* in wine samples will be presented.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
TRANSMITTAL COVER SHEET

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Commissioner for Patents, Washington, DC 20231 on this 17th day of January, 2002.

A handwritten signature in dark ink, appearing to read "Brian D. Gildea".

Brian D. Gildea
Reg. No. 39,995

Commissioner for Patents
Washington, DC 20231

Dear Sir or Madam:

Enclosed herewith, please find the following documents and sheets.

1. Response to the Office Action mailed on July 18, 2001.
2. Petition Under 37 C.F.R. § 1.136(a)
3. Declaration Under 37 C.F.R. §1.131.

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Attorney Docket No. BP9901-US

Respectfully submitted
On behalf of Applicants,

Brian D. Gildea 1/7/02
Brian D. Gildea; Reg. No. 39,995